DIFFERENTIAL SUSCEPTIBILITY AS A BASIS FOR MODIFICATION AND CONTROL OF EARLY DEVELOPMENT IN THE FROG.

A. W. BELLAMY.

CONTENTS.

I. INTRODUCTION AND GENERAL STATEMENT OF THE PROBLEM.

Observations on vertebrate teratology date almost from the beginning of descriptive biology, though active accumulation of experimental data on the frog especially, began little more than a quarter of a century ago. Among the early papers on the subject may be mentioned those of O. Hertwig (1892, 1894 1895, 1896); Gurwitsch (1895, 1896); Morgan and Tsuda (1894). These and other workers have studied the early development of certain teleosts, amphibia and birds under the influence of such physical factors as: gravity, mechanical pressure, temperature, light, electricity and magnetism, X-rays, radium emanations, atmospheric pressure, and a wide variety of chemical substances.

It is perhaps sufficient, here, merely to mention the essential similarity of terata produced by widely different methods, such, *e.g.*, as high and low temperatures, many different chemical agents, and heterogeneous hybridization. Equatorial gastrulation, embryos with permanent yolk plugs, with spina bifida, microcephalic forms and many other abnormalities in the frog have been produced by nearly all the methods known to experimental teratology. For instance, microcephalic frog embryos with permanent yolk plugs, and spina bifida have been produced by exposing different stages in development to such external agents as: high temperature (Jenkinson, 1909, p. 98, fig. 49); 0.6 per cent. NaCl (Hertwig, 1894, p. 315, 316, 1895, plate XX., figs. 5, 6, 7, 10, 12, 15, 16, et al); fertilizing "over-ripe" eggs (Hertwig, 1892); strychnine (Gurwitsch, 1896); sugar solutions (Bataillon, 1901, figs. 2, 5); Jenkinson (1906) produced these same types (among many others) in a number of isotonic solutions of Na_2SO_4, K_2SO_4, $NaNO_3$, urea, sugar solutions, et al.

Cyclopia in *Fundulus* has been produced by physical and chemical agents and by hybridization. Stockard (1909) obtained this anomaly with the aid of magnesium chloride and for a time ascribed a specific cyclopia producing property to this salt. Later, Stockard produced cyclopic monsters with alcohol and other substances and was forced to abandon his theory of specificity. McClendon (1912) had no difficulty in producing cyclopia in isotonic solutions of NaCl, LiCl, $MgCl_2$, NaOH and in alcohol, and other substances. McClendon is somewhat at a loss to explain his results but sums up the facts in these words: "We need only suppose that the cells between the eye anlagen are more easily affected than other cells of the embryo, to explain the action of the solutions." Werber (1915, 1916, 1917) obtained all the known types of terata in *Fundulus* by using substances usually found among the products of destructive metabolism, viz., butyric acid, acetone, urea, lactic acid, etc. In the concentrations used he found butyric acid and acetone, especially the latter, somewhat more effective than the other substances in the production of monstrous development. Werber looks upon abnormal development as due to a "blastolytic" action of the deleterious agent upon the primordial regions of the eggs, especially those at the anterior end of the body. Kellicott (1916) has secured the usual wide variety of terata in *Fundulus* simply by subjecting the early egg stages to the action of low temperature. He is inclined to look upon teratogenesis as being due in some way to a "disorganization" of the egg materials. Newman (1917) noticed the same range of abnormalities among his heterogeneous *Fundulus* hybrids and applied Child's axial gradient theory to the interpretation of these and other terata seen in teleosts produced by chemical and physical agents.

Perhaps enough has been said by way of a preliminary statement to indicate the essential similarity of experimentally produced monsters regardless of the methods employed in obtaining them. Likewise a little reflection on the various theories advanced to account for monstrous development makes apparent the lack, hitherto, of an adequate basis for a rational interpretation of them.

It is the purpose of this report to attempt an analysis of the problem of physiological axiation and teratogeny in the frog from a physiological point of view. It considers the general problem of physiological axiation, the origin of polarity, and presents data on the modification and control of early development in the frog on the basis of differential susceptibility.

II. Material and Methods.

1. *The Susceptibility Method.*—Observations on many species of animals and plants have shown that definite and characteristic differences in susceptibility to a wide variety of physical and chemical agents, within certain limits of concentrations or in-

tensities of action, exist in relation to the physiological axes.[1] These differences in susceptibility may be demonstrated in several ways: first, as differences in survival time of one region of the egg or embryo as compared to other regions, under conditions severe enough to kill without permitting acclimation to occur; second, as differences in the degree of inhibition of growth and development, or in certain cases as differences in the degree of acceleration of these processes; third, as differences in the rate or degree of acclimation to a certain range of less severe conditions; fourth, as differences in the rate or degree of recovery after temporary exposure to conditions that inhibit development.

These differences in susceptibility determined in these different ways are all expressions of the fact that a "differential susceptibility" to the action of external agents is a characteristic feature of physiological axes in both plants and animals so far as they have been examined with reference to this point. This "differential susceptibility" appears according to experimental conditions as differential disintegration associated with death, and as differential inhibition, acclimation or recovery in development.

The high degree of uniformity in the susceptibility relations in different organisms, both animals and plants, to a wide variety of agents and conditions, viz., cyanides, anesthetics, acids, alkalies, various salts, certain alkaloids, physical conditions such as extremes of temperature and certain conditions that may be termed negative, such as lack of oxygen, indicate that in their general features these susceptibility relations are independent of specific qualitative differences in the protoplasm of different forms. If this is the case they must depend upon quantitative differences of some sort which are common at least to all forms in which these differences in susceptibility have been shown to exist.

So far as present knowledge goes, the facts indicate that susceptibility, in the sense in which the term is used here, is

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[1] The extensive literature on this subject, previous to 1915, mostly the work of Child, is treated in two of his recent books (1915*a*, 1915*b*) to which the reader is referred for specific references. Other references are: Behre (1918); Child (1916*a*, 1916*b*, 1916*d*; 1917*a*, 1917*b*, 1917*c*, 1917*d*; 1919*a*, 1919*b*); Child and Hyman (1919); Hyman (1916*a*, 1916*b*; 1917*a*, 1917*b*; 1919).

associated in some way, directly or indirectly, with the rate of certain fundamental metabolic reactions, such, *e.g.*, as oxidation-reduction processes, and the protoplasmic conditions associated with them. Considerable evidence has been accumulated which indicates that in general the susceptibility to lethal or strongly toxic conditions and the capacity for acclimation to less severe conditions and for recovery after temporary exposure—all vary more or less directly with, though not necessarily proportionally to, the rate of fundamental metabolic reactions. This does not mean that all the agents employed act directly upon these reactions in every case or that all act in the same way upon protoplasm. It probably means merely that living protoplasm is a system of more or less closely associated and interdependent reactions and conditions so that no essential factor in this system can be altered beyond a certain degree without involving the system as a whole.

But, however the facts of differential susceptibility may finally be interpreted, concerning the facts themselves there can be no doubt. They have afforded a means not only of demonstrating characteristic differences in physiological conditions along the axes of organisms but also of modifying and controlling development in definite ways through these differences. This study of the development of the frog was undertaken in the attempt to determine whether, and to what extent, the susceptibility method could be used in modifying and controlling development in a vertebrate, as they have been used in investigations of the physiology of development in certain invertebrates.

I am under obligation to Professor C. M. Child, in whose laboratory this work was done, for essential aid in the way of suggestion and criticism, and to Dr. L. H. Hyman for many thoughtful suggestions. Work on amphibia involving the susceptibility method was begun by Dr. Child in 1913. In the spring of 1916, work was begun by him on the modification and control of development in the frog, on the basis of differential susceptibility; the data and preserved material of these experiments were turned over to me late in that year. My own work was done in the spring of 1917 and the spring of 1919 during which time approximately one hundred thousand eggs were handled, of which about forty thousand were preserved.

2. *Routine and Methods of Handling Eggs.*—Clasping frogs (*Rana pipiens*) were obtained from a local dealer and placed in a large cement aquarium arranged to simulate natural conditions as nearly as possible. The temperature of the water was kept between 5° and 9° C. There was no difficulty in obtaining all the eggs that could be used; and with a little patience they could be had as soon after deposition as desired. As many as five females have been seen spawning at one time. Shortly after deposition each egg mass was given a number, the jelly cut into bits containing ten to twenty eggs each, and then placed in a gallon jar of the cold aquarium water. About an hour before being introduced into experimental conditions the jar of eggs was removed to the laboratory to warm slowly up to the room temperature (17 ± 1° C.).

For the modification of development the following chemicals were used: potassium cyanide, formaldehyde, potassium permanganate, mercuric chloride, magnesium chloride, lithium chloride, hydrochloric acid, sodium hydrate, and ethyl alcohol. The method of handling the eggs varied somewhat with the chemical used. Eggs treated with KNC, CH_2O, HCl, NaOH and C_2H_5OH, were placed in liter Erlenmeyer flasks filled almost full and stoppered. For the other chemicals, glass finger bowls of 300-c.c. capacity, one- and two-liter candy jars were used and covered with glass plates to prevent evaporation.

In a given experimental series similar glassware was used, each vessel containing approximately the same number of eggs from the same female. In every case one vessel of the series was used as a control and was treated in the same way as regards stoppering, changing of fluids, etc. Eggs from the same females developing in liter Erlenmeyer flasks filled nearly full and stoppered, and in the same volume of water in open dishes did not differ in rate or manner of development up to some time after hatching, provided the water was changed once in twenty-four hours and when there were not more than 100 c.c. of egg mass in the stoppered flask. Especial care was taken to use no more than 15 to 20 c.c. of egg mass in stoppered vessels, and to change solutions in all experiments daily.

As will be noted later, the frog egg undergoes a tremendous increase in susceptibility during the early stages of development, and to provide for this and to carry the effects of differential inhibition somewhat farther than is possible where the eggs remain in the same concentration throughout the experiment, a number of experiments were done where the solution was gradually diluted as development proceeded.

In the experiments on recovery, the eggs were removed from the solutions and washed in several changes of water to remove any of the chemical remaining in the jelly.

III. The Origin of Polarity.

1. *Polarity in Other Organisms.*—There is abundant evidence to show that for many organisms and especially among the plants, polarity and symmetry arise in response to external conditions. Polarity in the egg of the alga *Fucus*, and in the spore of *Equisetum* is usually determined by the direction of incident light. In some algæ, polarity and symmetry are directly under the influence of light, even in the vegetative thalli, and are reversible or modifiable by change in light relations. In various liverworts and free prothallia, and even in certain phanerogams, light is an important factor in determining dorso-ventrality in branches.

The polarity of a number of plant and animal eggs bears a definite relation to their manner of attachment to the parent body during growth stages. In the phanerogams, for example, the free end of the egg becomes the apical, the attached end the basal end of the plant axis, and the same relation holds for a number of the lower animals.

Likewise a polar axis of symmetry once established may be obliterated experimentally and a new one induced by conditions external to the organism. This has been done by H. V. Wilson (1907, 1911) in certain sponges and hydroids, work which was confirmed by Hargitt (1915). In experimentally produced biaxial forms in planaria, hydroids and annelids, a new axis arises 180° from the old one. In *Corymorpha, e.g.*, Child showed that when pieces of the stem are placed in 2 per cent. to 2 1/2 per cent. alcohol in sea water, "in the course of a few days the pieces become shorter and more rounded, decrease in size, and lose the characteristic structure of the *Corymorpha* stem." On removal to water after several days in alcohol, a new individual arises from the old tissue with its axis at right angles to the old one.

Various investigators have observed that under certain conditions, *Hydra* may lose its characteristic structure and "melt" down into a shapeless mass from which a new individual may arise. Dr. Hyman, in this laboratory, has recently observed that such masses often give rise to several hypostome regions each with tentacles.

Physiological axiation begins, of course, with the origin of polarity in the egg. Polarity may, conceivably, arise in several ways. First, axiation and polarity may, as has been commonly supposed, exist in protoplasm in relation to some intrinsic molecular or other structure which is a fundamental property of living matter; second, the primordial cell, from which the ovum arises, may inherit its polarity, which, if true, merely pushes the problem farther back; third, the appearance and position of local differences in structural and functional order in the egg protoplasm, that constitutes polarity, may arise during the growth and development of the egg in response to conditions external to it. The first alternative has been shown to be untenable, and the second offers so little satisfaction, that, in the light of the accumulated evidence, it seems necessary to consider the last alternative as the more probable one, at least until it has been shown that polarity arises in some other way.

2. *Origin of Polarity in the Frog's Egg.*—The mature egg of the frog is described as possessing a radial symmetry about an imaginary axis, the polar axis, passing approximately through the centers of the pigmented (animal, apical) hemisphere and of the unpigmented (vegetative, basal) hemisphere. This polarity is marked by the localization of most of the protoplasm in the pigmented hemisphere, by the eccentricity of the nucleus, and by the distribution of pigment. In order to determine, if possible, whether, in the frog egg, a relation exists between its polarity and conditions external to it, a study was made of the ovarian relations of the egg, especially of the relation of the polar axis to the region of attachment to the ovarian membrane and to the blood supply of the egg.

Some days or weeks before extrusion, the egg is suspended from the ovarian membrane in an epithelial sack (Fig. 1). This sack, the theca, forms at one region on the egg a narrow stalk, the pedicle, which becomes continuous with the ovarian membrane. Beneath the theca is a layer of follicular cells and beneath this, the vitelline membrane. In the theca are found the blood vessels that supply the egg.

Table I. gives the location of the pedicle with reference to the distribution of the pigment on the egg. It will be noted that in approximately eighty per cent. of the cases, the region of attachment (the pedicle) is near or on the boundary between the pigmented and unpigmented regions of the egg (Fig. 2).

Table I.

Location of Pedicle.	Number of Eggs Observed.	Per Cent.
Position A (see Fig. 2)	75	12.2
Position B	217	35.3
Position C	259	42.2
Position D	63	10.3

These results were obtained by taking a number of eggs at random from various parts of the ovary and determining the location of the pedicle for every egg in each sample.

A number of specimens were injected through the conus arteriosus with a lead chromate-starch-gelatin mass[1] and in the great majority of cases only those vessels lying over the unpigmented hemisphere remained uninjected and could often be traced by the color of the blood remaining in them. In several cases injected vessels were seen to pass a little distance over the unpigmented hemisphere, but in no case was an egg observed where more than a small per cent. of the vessels overlying the yolk were injected (Fig. 3). Several unsuccessful attempts were made to inject the venous system.

In order to eliminate any error due to the injection mass passing through the capillaries into the veins, several frogs were opened under normal salt solution and the circulation of the blood over the egg determined with the aid of the binocular microscope. In every case where the movement of corpuscles over the pigmented hemisphere could be seen, the circulation

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[1] Guyer (2d edition, page 84). The mass was made up in a 1 per cent. uncooked starch suspension, warmed and well stirred immediately before the injection was made.

was away from the heart and therefore arterial. The movement of blood over the unpigmented hemisphere, where the movement of corpuscles could be seen more clearly than over the pigmented hemisphere, was always toward the heart and therefore venous. As was noted for the injected specimens, a small per cent. of the eggs showed arteries passing a little distance over the yolk. But it is especially to be noted that in every case observed, the greater part of the arterial blood supply was restricted to the pigmented hemisphere.

In the ovary of the frog, the polar axes of the eggs lie in every possible direction with respect to gravity, so that the possibility of polarity being determined in the ovary with respect to gravity is at once eliminated.

The data indicate that polarity in the frog egg arises at some time during the growth stages, in response to external conditions, viz., to the blood supply of the egg: that region of the oögonium chancing to be most richly supplied with arterial blood being destined to become, by virtue of this respiratory and nutritive relation, the animal pole of the egg. Naturally, the region on the surface of the egg where the capillary net work is most extensive would be effective in determining polarity rather than the point where the blood vessels enter the theca.

A region of higher oxidation rate in the egg, or at any rate the proximity of this region to a greater oxygen supply (arterial blood) is further evidenced by the appearance of pigment—a melanin (Kellicott)—over a limited surface of the egg, for it is well known that oxygen is necessary for the formation of these pigments. It is probable that the origin of polarity and the appearance of pigment in a symmetrical relation to that polarity are both expressions of the localized oxygen supply (or nutritive supply, or both) of the egg.

It may be noted here also that pigment appears most densely in the most active regions of the egg where other evidence indicates that oxidations are proceeding more rapidly than elsewhere. In other words, the density of pigmentation seems to be an expression of the rate of at least certain oxidations occurring in that region. Local increase in activity results in the formation or increase of the pigment and local decrease in activity results in diminution of pigment. Those unpigmented cells lining the archenteron in the early gastrula stages, *e.g.*, become pigmented and most densely where cell division is most rapid.[1] And to anticipate somewhat, the subjection of early gastrula stages to strongly inhibiting conditions, results in a marked diminution of pigment in the dorsal lip regions.

The data further indicate that the polar axis as well as other axes that arise later in development are primarily gradients in fundamental physiological conditions or processes, which are made evident in several ways, such as, the differential cleavage rate which results in a gradient in cell size, by gradients in susceptibility, etc. (see pp. 322–328).

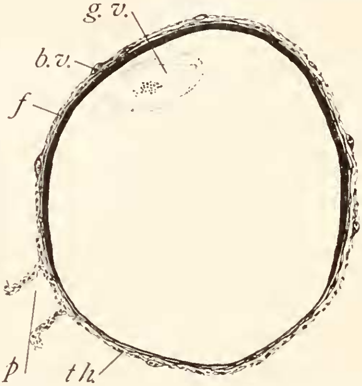

FIG. 1. Semi-diagrammatic view of a section through the germinal vesicle and pedicle of a full grown ovarian egg. *b.v.*, blood vessel in the theca; *f*, follicle cells; *g.v.*, germinal vesicle; *p*, pedicle; *th.*, theca.

Discussion of the question of whether the location of the pedicle and the localized blood supply is related in any way to the definitive bilaterality remains for future consideration.

The fact that the pedicle is equatorial in position is of course, merely a necessary consequence of the way polarity and the accompanying distribution of pigment is determined by the blood supply.

IV. EXPERIMENTAL DATA.

1. *Susceptibility to Lethal Concentrations of External Agents.*

The early stages of the frog egg are extremely resistant to toxic agents. Eggs just beginning to segment, when placed

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[1] King (1902).

e.g., in *m*/10,000 $HgCl_2$, are accelerated in development for a short time, while gastrulating eggs die within a few hours (four to six) in *m*/2,000,000 $HgCl_2$, without having developed farther after being placed in the solution. The same relation holds for other agents. Eggs from different females may differ considerably in their behavior when exposed to toxic agents; also eggs from the same female may differ somewhat in susceptibility to toxic agents but to a much less extent than in the former case. Some difficulty is experienced in watching the progress of disintegration in early stages, especially in concentrations that kill rapidly, on account of the spreading of the disintegrated protoplasm underneath the inner membrane, and thus obscuring the rest of the process. However, the progress of disintegration, wherever observed, is an orderly process and bears a definite relation to the polar axis of the egg and to the axes of symmetry of the embryo.

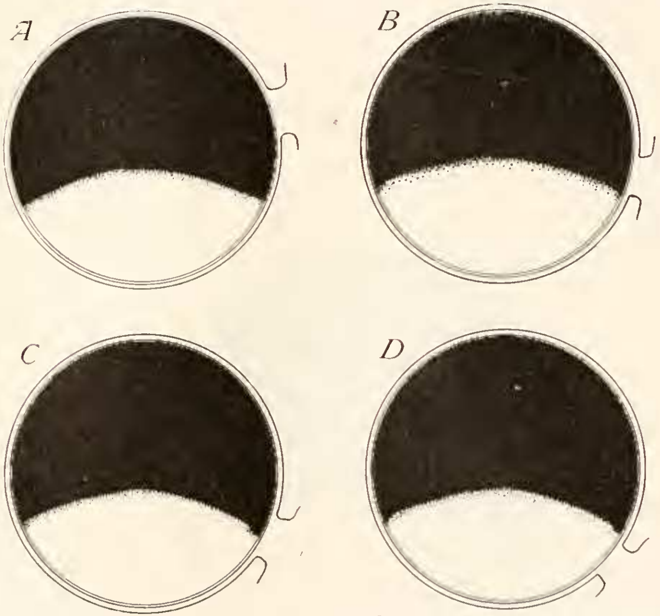


FIG. 2. Diagrams of full grown ovarian eggs to show the position of the pedicle in relation to the distribution of pigment. (See also Table I.)

(*a*) *Unsegmented Eggs.*—The most satisfactory method found for observing disintegration in the unsegmented egg, was to expose the egg soon after deposition, to a temperature of 0° C. for ten days or two weeks. Fig. 4 illustrates several different stages in the process of disintegration under such conditions. The eggs were deposited in the aquarium (temperature, 6.5° C.) March 26, 9:00–9:15 A.M., and were placed in the refrigerator at 10:00 A.M. On April 7, the eggs were in the condition shown in the figures. The relation of the disintegrated areas to the grey crescent and to the polar axis is obvious.

(*b*) *Cleavage Stages.*—The process of disintegration during early cleavage has been studied at low temperature, in KNC, *m*/1,000; *m*/100; $HgCl_2$, *m*/10,000; in alcohol, 5 per cent., 8 per cent.;

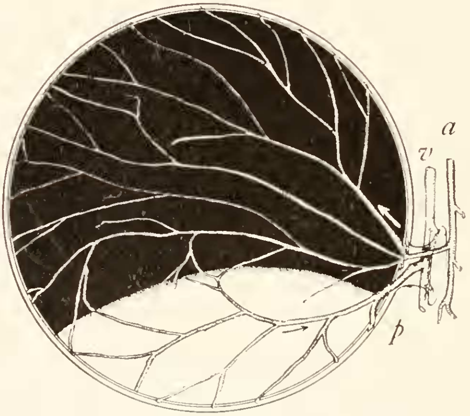


FIG. 3. Semi-diagrammatic view of ovarian egg showing the distribution of arteries and veins. The blood vessels were outlined under the camera lucida.

and incidentally in all of the other agents used to modify development. The process of disintegration in all of these cases is essentially similar, except in the case of alcohol, which has a marked solvent action on the yolk. In any concentration of alcohol above 1 per cent., the yolk becomes rough and pitted, an appearance that is much accentuated in the higher concentrations (5 per cent. to 8 per cent.). Disintegration usually begins in a meridian that bisects the grey crescent and near the center of the pigmented hemisphere. The surface membranes of individual cells in this region break down and turn white. Shortly afterward, disintegration begins in the equatorial region just above the middle of the grey crescent and spreads equatorially and apically to join the area where dissolution first began. At this time the disintegrated area forms, roughly, a triangular figure whose apex is the point where disintegration first began, and whose base is the equatorial region immediately above the gray crescent. Disintegration then spreads more or less symmetrically from the apex and sides of the triangle over the rest of the animal pole, and from the base to involve the grey crescent region. Fig. 6 illustrates the process just described. The history and treatment of the eggs is given in the legends to the figures.

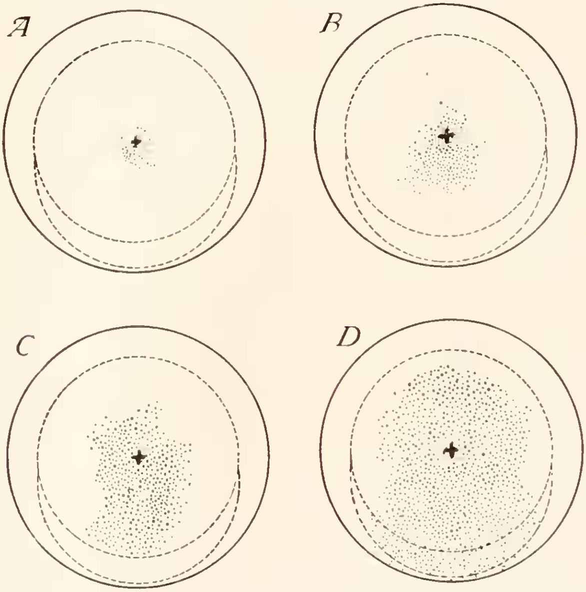


FIG. 4. Unsegmented egg seen from above (apical pole) showing different stages in disintegration after twelve days' exposure to a temperature of 0° C. Disintegrated areas are represented by stippling. The dotted circle represents the boundary between pigmented and unpigmented hemispheres. The dotted crescent outlines the gray crescent. The cross marks the animal pole.

(*c*) *Gastrula Stages.*—Eggs in an early gastrula stage, when placed in lethal concentrations of a toxic agent, always begin to disintegrate first in the dorsal lip region, and shortly afterward in the same meridian about 120° to 130° above the blastophore. From this upper point dissolution of the surface cells proceeds down the meridian and meets the disintegrated area of the dorsal lip region, which has spread apically and now includes the lateral lips. The area then spreads more or less symmetrically from the apical and lateral borders of the disintegrated area until all of the pigmented cells are involved. The yolk cells retain their structure long after the pigmented cells have completely disintegrated, except in the case of alcohol, which attacks the yolk rapidly from the start. Fig. 8 illustrates the general process.

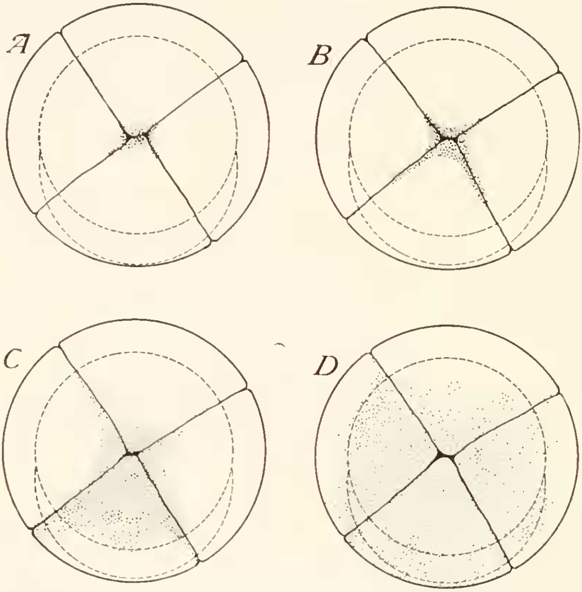


FIG. 5. View from animal pole showing stages in the disintegration of eggs in a four-cell stage after ten days' exposure to a temperature of 0° C. The eggs were in a two-cell stage when placed in the cold chamber.

(*d*) *Later Embryonic Stages.*—With the beginning of elongation, while two regions of high susceptibility are still present, viz., the apical and the dorsal lip region (now posterior), the posterior growing region, especially during the formation of the neural folds, appears relatively less susceptible than it did earlier. This apparent greater difference is probably due to local differences associated with the formation of the neural plate. In embryos beginning to elongate, disintegration begins first at the apical end in the medial dorsal region and spreads laterally and posteriorly, more rapidly in the posterior direction, to meet an area of disintegration that has just begun in the region where the tail bud appears later. In neural fold stages disintegration begins at the anterior end of the floor of the neural groove usually at two points on either side of the median line, where the primordia of the optic vesicles have appeared. Often, and especially in earlier neural fold stages, the disintegration begins in the median line at the anterior end of the neural groove. From this point or points as the case may be, disintegration spreads back along the neural groove nearly to the posterior end, where it joins an area that has just begun to undergo dissolution. In the meantime, the medullary folds, ventral suckers, and anterior part of the head region have begun to disintegrate.

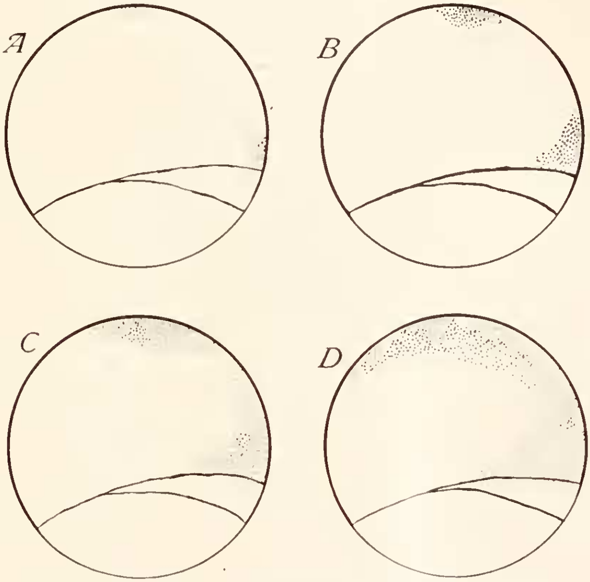


Fig. 6. Disintegration stages during late cleavage. The eggs are shown in side view with the animal pole uppermost and the gray crescent to the right.

With the closure of the neural folds and the further elongation of the embryo, local differences in susceptibility begin to appear with the differentiation of certain organs. While in general, the apical-medial-dorsal region begins to disintegrate first, the dissolution of cells in this region may be followed shortly, or even preceeded by, the disintegration of rapidly proliferating cells in certain regions of the embryo, such, *e.g.*, as the tail bud, optic vesicles, nasal pits, ventral suckers, and other rapidly growing regions.

These data on disintegration are in agreement with the data on differential inhibition, acclimation, and recovery, in showing that certain parts of the egg and embryo are more susceptible than other parts, and that these differences in susceptibility have a definite relation to the polar axis and to the plane of bilaterality in the early cleavage stages and to other physiological axes arising later in development. The significance of differential susceptibility is discussed later (pp. 346–349).

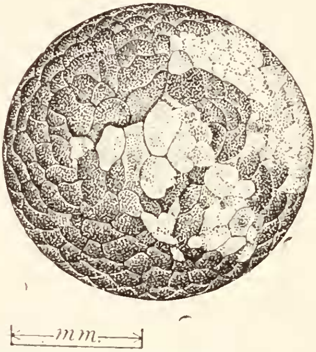


FIG. 7. Camera lucida drawing of an egg showing disintegrated cells (white) following 24 hrs. exposure to $m/1{,}000$ KNC and 24 hrs. to $m/5{,}000$ KNC, from a two-cell stage. The animal pole is in the center of the figure. (Experiment KNC, B3.)

2. *Experimental Modification of Development.*

For the sake of convenience in description, certain arbitrary terms have been used to designate different "types" of abnormalities. The differential inhibition of the cleavage ratio in early development $\left(\frac{\text{size of animal pole cells}}{\text{size of vegetative pole cells}}\right)$ is expressed as a fraction whose denominator is ten. For example, the expression "cleavage = 7/10" means that the size of the animal pole cells is to the size of the vegetative pole cells, as 7 is to 10. The expression "V-shaped blastopore" refers to a condition where the blastopore takes the shape of an inverted "V" or "U," and is intended to describe conditions where the dorsal lip is retarded to a relatively greater extent than the lateral lips (Fig 14, *A*). The term "wide-crescent blastopore" refers to a condition where the blastopore is in the form of a broad crescent (Fig. 17), and is intended to describe a condition where both dorsal and lateral lips are inhibited. The term "secondary invagination" describes a situation where a second infolding takes place apical to an original equatorial blastopore (Fig. 18). The "gastrular angle" is the angle between a plane through the center of the egg parallel to the floor of the segmentation cavity, and a line through the center of the egg and the blastopore (Fig. 9). Obviously, comparison of the gastrular angle of normal eggs with this angle in inhibited eggs is possible only at the *beginning* of gastrulation.

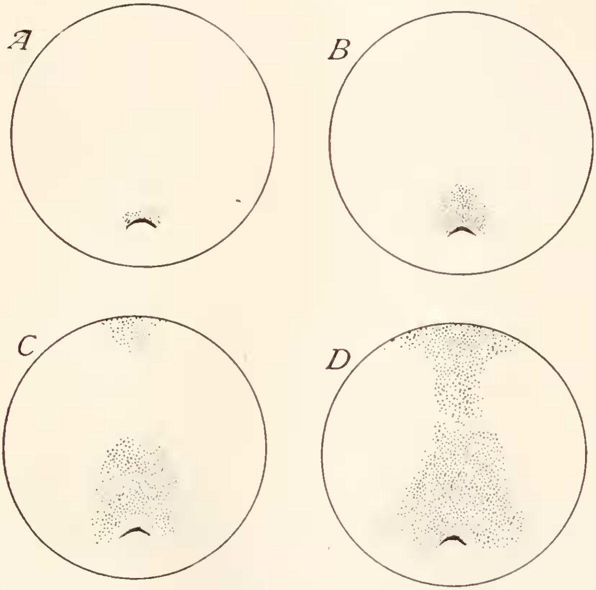


FIG. 8. Stages in the disintegration of eggs in an early gastrula stage following several hours exposure to $m/1{,}000$ KNC, or in $m/1{,}000{,}000$ $HgCl_2$. The progress of disintegration follows this general plan in all agents in concentrations that kill the egg within several hours.

The development of the frog is so well known that a detailed description of the process is unnecessary. However, mention of several features of normal development will be of use for comparison with abnormal types.

Cleavage begins first and proceeds more rapidly in the pigmented hemisphere than in the vegetative hemisphere, establishing early in development an inequality in the size of the cells in the two hemispheres. In an early cleavage stage, the size relation of the cells in the two regions is in the proportion of 4/10 to 5/10, a ratio that decreases in value up to about the time of gastrulation, when the ratio may be represented by 2.5/10 to 3.5/10. During mid-cleavage stages the cell size ratio is approximately 3.5/10 to 4/10. This does not take into account those pigmented cells on the grey crescent side of the egg, which are slightly smaller from the beginning of cleavage than the other pigmented cells (Morgan and Boring, 1903). Immediately preceding the appearance of the blastopore, those cells in the dorsal lip region are smaller than any of the other surface cells.

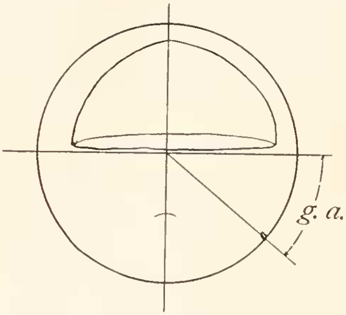


FIG. 9. Diagram to illustrate the gastrular angle, *g.a*

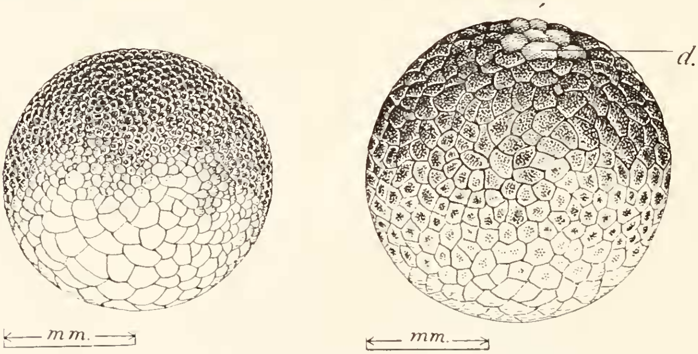
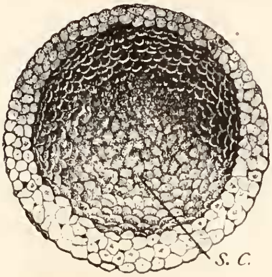


FIG. 10. *A*, control egg with a cleavage ratio of approximately 3.5/10–4/10. *B*, egg from the same batch as the one in Fig. 10, *A*, in which the cleavage ratio approximates 10/10. Same treatment as egg illustrated in Fig. 7. (Experiment KNC B 3.)

The gastrular angle at the time of the appearance of the blastopore approximates 40°.

In addition to the indications of bilaterality by the appearance of the grey crescent, bilaterality is made more apparent during the blastula stages by the movement of material from the pigmented region toward the equator—a process that takes place more rapidly in the sagittal plane than elsewhere, and results in the thinning out of the walls and roof of the segmentation cavity, which now comes to lie nearer that side of the egg where the dorsal lip appears. The so-called germ ring, formed by the downward movement of animal pole material, and which lies at first on the equator and later below it, extends somewhat farther down on the egg in the dorsal lip region. To express the situation in other words: growth in length (between the apical



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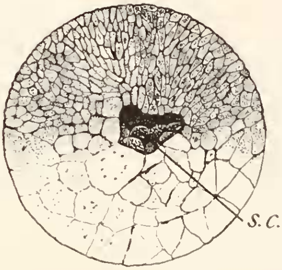

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FIG. 11. Enlarged segmentation cavity. Eggs in beginning two-cell stages treated as follows with KNC: $m/1{,}000$ 24 hrs., $m/5{,}000$ 24 hrs., $m/10{,}000$ 12 hrs., $m/20{,}000$ 12 hrs. (Experiment KNC H 5.)

FIG. 12. Small segmentation cavity as seen under conditions of severe inhibition. In this case eggs were exposed 48 hrs. from a two-cell stage to $m/10{,}000$ $HgCl_2$. (Experiment IV 40.)

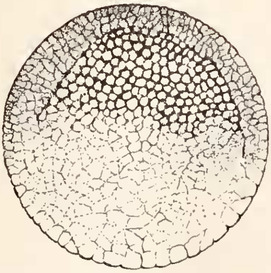

FIG. 13. Obliterated segmentation cavity. (Experiment IV 56.)

pole and the germ ring) is greater and proceeds most rapidly in the sagittal plane. The significance of this process is discussed elsewhere (p. 349).

A. *Disturbances in Cleavage such that the Egg Never Gastrulates.* (*a*) *Cell Size Ratios.*—In frog eggs so strongly inhibited that they do not gastrulate, cleavage usually begins and proceeds more or less normally for several hours. The rate of cleavage in the animal hemisphere then becomes less and less rapid, relatively, until the cell size ratio approaches and may become equal to one (10/10, Fig. 10, *B*), whereas this ratio in a normal egg of a comparable stage of development is about 3.5/10 to 4/10 (Fig. 10, *A*).

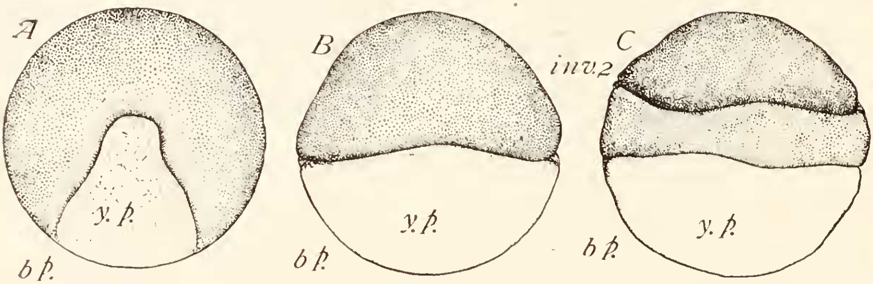

FIG. 14. V-shaped blastophore, *A*; Equatorial gastrulation, *B*; Secondary invagination, *C*. Produced in m/500,000 $HgCl_2$. About 40 per cent. of the eggs showed the V-shaped blastophore. (Experiment IV 70.)

The cleavage ratio was found to increase in value consistently in all cases[1] where inhibition was sufficient to prevent the com-

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[1] This distortion of the cleavage ratio was obtained in: Experiments KNC A 1; H 1; exposure 12 hours in m/1,000 from 2-cell stage; cl. = 5/10 to 7/10. Experiment *KNC—C.7;* exposure 24 hours in m/2,000 from 2–4-cell stage; cl. = 7/10. *Experiment KNC—C.8;* 48 hours exposure to m/2,000 from 2-cell stage; cl. = 8/10. Experiment *KNC—C.12;* 48 hours exposure to m/2,000 from 2-cell stage; cl. = 7/10 to 10/10. Experiment KNC—C 1; 12 hours exposure to m/5,000 from 2-cell stage; cl. = 5/10 to 6/10. *Experiment KNC—C.6;* 24 hours exposure from 2–4-cell stage; cl. = 5/10 to 7/10. Experiment *KNC—C.9;* 5 days exposure to m/10,000 from 2 cell-stage; eggs beginning gastrulation (equatorial) with 6/10 to 8/10 cl. Experiment IV 21; 21 1/2 hours exposure to m/8.5 LiCl from an unsegmented stage; in about 15 per cent. to 20 per cent. cl. = 4/10–7/10. Experiment IV 31; unsegmented eggs exposed 4 hours in m/4 LiCl, then 7 1/2 hours in water showed an 8/10 to 10/10 cleavage ratio. Experiment IV 54; eggs in late cleavage exposed 40 hours to m/100,000 $NgCl_2$; cl. = 6/10 to 10/10. Experiment IV 62; eggs in 4–8-cell stage exposed 12 hours in 0.0075 per cent. formaldehyde; cl. = 6/10 to 10/10. Citations of experiments in italics are from Dr. Child's preserved material. "cl." = cleavage ratio.

pletion of gastrulation, although the proportion of eggs showing this type of differential inhibition varied somewhat in experiments involving different chemicals. This increase in value of the cleavage ratio appears less frequently in LiCl, $MgCl_2$, and in alcohol, and most frequently in KNC and formaldehyde. In *m*/1,000, *m*/2,000 KNC, fully 90 per cent. of the eggs are of this type after 24 to 36 hours' exposure from the beginning of segmentation. Differential inhibition under the conditions stated, is diagrammatic, especially in KNC, and represents simply a greater susceptibility to the toxic agent, of the cells most affected, viz., the animal pole cells. The significance of this differential susceptibility is a matter of discussion elsewhere (pp. 346–349).

(*b*) *Bilaterality.*—Under the conditions of these experiments, bilaterality is much obscured and may not become evident at all (Fig. 20). But, that bilaterality is at least potentially present is indicated in those cases where gastrulation does begin (always equatorially) the wrinkling and folding, indicating the initiation of gastrulation usually begins at a definite point on the equatorial region and spreads equatorially around the egg. A few cases have been observed in *m*/5,000 KNC where the equatorial folding occurred almost simultaneously around the entire equator of the egg.

(*c*) *The Segmentation Cavity.*—Under severe inhibiting conditions, the segmentation cavity shows several characteristic consequences of differential inhibition, depending chiefly upon the severity of the conditions and the stage at which the eggs are exposed to them and upon the length of the exposure.

Some of the data are these: In eggs exposed to *m*/1,000 or *m*/5,000 KNC (Experiments KNC A–H) from the beginning of the first segmentation, development usually ceases in late cleavage stages with occasional abortive attempts at equatorial gastrulation. The yolk cells, which are relatively less inhibited, may continue division for a time after it has nearly or quite stopped in the animal hemisphere. In such cases, one finds that the more peripheral yolk cells, forming the floor of the segmentation cavity, proliferate apicalward, forming a thin layer of yolk cells that may partly or completely line the walls of the segmentation cavity—which becomes very large. At the time of death, the egg resembles in some respects an amphioxus blastula (Fig. 11). Superficial indications of bilaterality are obscure.

Where inhibition is more severe, *e.g.*, in $m/10,000$ $HgCl_2$ (Experiment IV 40), eggs exposed to the solution for 48 hours from a two-cell stage, stop development in late cleavage stages. Gastrulation never occurs. In all of the eggs, the downward migration of materials from the apical pole is completely inhibited and the walls of the very small segmentation cavity lying near the center of the egg, are 2/5 to 4/9 the diameter of the egg in thickness (Fig. 12).

The partial or complete obliteration of the segmentation cavity may occur in an entirely different way under less severe inhibiting conditions. Eggs in late segmentation stages, exposed 15 minutes to $m/10,000$ $HgCl_2$ (Experiment IV 56) then returned to water, show after three days, the segmentation cavity completely filled with small yolk cells that have proliferated from the floor of the cavity. A faint line indicates the walls of the now obliterated cavity. The walls of what was the segmentation cavity are much thinner than when the eggs had been exposed 48 hours to $m/10,000$ $HgCl_2$ from the beginning of cleavage.

Likewise in $m/10$ LiCl, where development proceeds abnormally to the time of hatching, the proliferation of yolk cells from the margin of the floor of the segmentation cavity may be so extensive as to fill it completely.

All of these different modifications appear to be simply different expressions of the differential inhibition which the eggs have suffered. The animal pole cells being most susceptible, are most affected by the adverse conditions, the yolk cells least.

Those small cells around the margins of the floor of the segmentation cavity, which under certain conditions of inhibition, continue division and finally fill the blastocoele completely, appear to be the ones destined to give rise to mesoderm (Morgan, 1906, p. 129; Kellicott, 1913, p. 107; King, 1902, Fig. 4). It is significant here that in the sea urchin Child (1916, p. 91) found the mesenchyme cells which arise from the basal pole of the egg, to be less susceptible than other parts of the egg, and that under conditions of differential inhibition, these cells tended to "run wild" and result in an excessive over-development of skeletal structures.

(*d*) *Meroblastic Cleavage.*—In exceptional cases, and under the influence of conditions so severe that development stops completely in early cleavage, cell division may be partly or completely restricted to the animal pole. This type of cleavage has been observed following exposure: to urea, 2.34 per cent. (Jenkinson, 1906, Figs. 38, 41); to NH_4I (Jenkinson, 1906, Fig. 32); to Na_2SO_4 (Jenkinson, 1906, Fig. 40); to temperatures above 26° C. (Hertwig, 1895), et al. In my own experiments it has been observed among eggs exposed six to ten hours to 2 per cent., 3 per cent. alcohol, a result that is not surprising when one considers the solvent action of alcohol upon lipoid substances generally. Four factors seem primarily concerned in the production of these restricted cleavages, viz., time, concentration or intensity of action of the agent used, physical effect of the particular agent to which the eggs are exposed, and the stage at which the eggs are exposed to the agent. So far as my observations go, this type of cleavage, rare at best, is realized only when the eggs are exposed to the inhibiting conditions at the time of, or immediately preceding the appearance of the first cleavage plane. If the eggs are exposed to concentrations necessary to the production of this type of cleavage, several hours before cleavage would normally begin, they never segment. The physical effect of alcohol has been mentioned. The time element is more or less obvious. Cleavage begins first, *normally*, in the pigmented hemisphere, and under the conditions necessary for the production of meroblastic cleavage the first cleavage plane makes its appearance, in all probability, before the effect of the inhibiting conditions (in the case of chemical substances particularly) penetrate the gelatinous membranes of the egg.

B. *Disturbances in Gastrulation.*—The various modifications of the process of gastrulation, following inhibition, fall into several more or less distinct groups, the particular types obtained depending largely upon the severity of the inhibiting conditions and upon the stage at which the eggs are introduced into the experiment; *i.e.*, depending upon the treatment and the physiological condition of the eggs. Individual variation is an important limiting factor in the experimental control of modifications. The range of variation in this respect seems to be widest during the early cleavage stages. In the great majority of experiments, while one usually finds a complete series of stages from nearly normal to the most extreme deviations from the normal, the modifications produced tend to conform to certain types, varying with the physiological condition of the eggs and the treatment they receive. It is therefore necessary to speak in terms of averages in discussing a given type of abnormality.

In general, as the concentration of a given chemical to which the eggs are exposed, is increased, or with longer exposure to the same concentrations, the modifications produced become more extreme. They are discussed in the order in which they appear.

(*a*) *Retardation of the Dorsal Lip Region.*—These are stages in which only the dorsal lip region seems much inhibited, although occasionally the cleavage ratio may increase in value somewhat, and the gastrular angle (see p. 329) in a small per cent. of the cases may be slightly more acute than in the control eggs. Where only the dorsal lip region is inhibited, the blastopore takes the form of an inverted V or U and when complete the yolk plug is oval, and may be several times longer than broad in the sagittal plane.

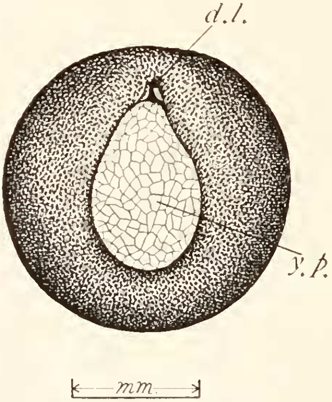

FIG. 15. Inhibition of the dorsal lip region after 38 hours exposure to *m*/10.62 LiCl. Eggs placed in the solution were in 16–32-cell stages—24 hours after deposition. (Experiment IV 76.) *d.l.*, dorsal lip region; *y.p.*, yolk plug.

Experiments IV 70; G, H, I. Eggs deposited March 28, 3:00 P.M. Removed to the laboratory 4:00 P.M. Introduced into $m/500{,}000$ $HgCl_2$, 5:00 P.M.—unsegmented at this time. After 24 hours the eggs were washed and returned to water. Twenty-four hours later (March 29, 5:00 P.M.) the stages shown in Fig. 14 were recorded. About 40 per cent. of the eggs showed the V-shaped blastopore (Fig. 14, *A*). Eggs in the three experiments were alike and among them everything was present from a few with closed blastopores to several forms showing a secondary invagination midway between the original (equatorial) blastopore and the apical pole. The equatorial gastrulæ resemble those produced in $m/10$ LiCl.

Experiment IV 91. Unsegmented eggs, placed in $m/2{,}000{,}000$ $HgCl_2$. Forty hours later, the eggs were mostly in inhibited gastrula stages (V-shaped blastopores).

Eggs introduced into $m/10.62$ LiCl during 16–32 cell stage, exhibit the inverted V- or U-shaped blastopore after 24 hours' exposure (Experiment IV 76). Eggs introduced into $m/10$ LiCl immediately before gastrulation (Experiment IV 59) are in gastrula and large yolk plug stages after eighteen hours in the solution. The majority of gastrula stages show the long inverted V-shaped blastopore and in those eggs showing yolk plugs, there is a tendency for the yolk plugs to be much larger than normal and somewhat oval in shape (Fig. 16).

After 48 hours in the solution, ninety per cent. of the eggs were in early neural fold stages, with much elongated yolk plugs.

In experiment IV 62 unsegmented eggs, five hours after deposition, were introduced into 0.0075 per cent. formaldehyde, and varied from equatorial gastrulæ to early neural fold stages after sixty hours' exposure. A few showed secondary invaginations.

In experiment R 138 a, eggs in early gastrula stages were placed in $m/1{,}000$ KNC. After a 12-hour exposure, 90 per cent. of the eggs showed a long inverted V-shaped blastopore or much elongated yolk plug. The eggs did not develop farther in this solution.

(*b*) and (*c*) *Retardation of Both Dorsal and Lateral Lips of the Blastopore, and Equatorial Gastrulation.* Eggs showing retardation of both dorsal and lateral lips are described as "flat-crescent" gastrulæ, for the reason that under the slightly more severe inhibiting conditions where both dorsal and lateral lips are inhibited, the blastopore forms, when about half completed, a figure like a much flattened crescent. When such a blastopore is complete the yolk plug is always much larger than in the control. All stages exist between this flat crescent type and those cases where the blastopore extends around the equator of the egg (equatorial gastrulation). In these forms the cleavage ratio

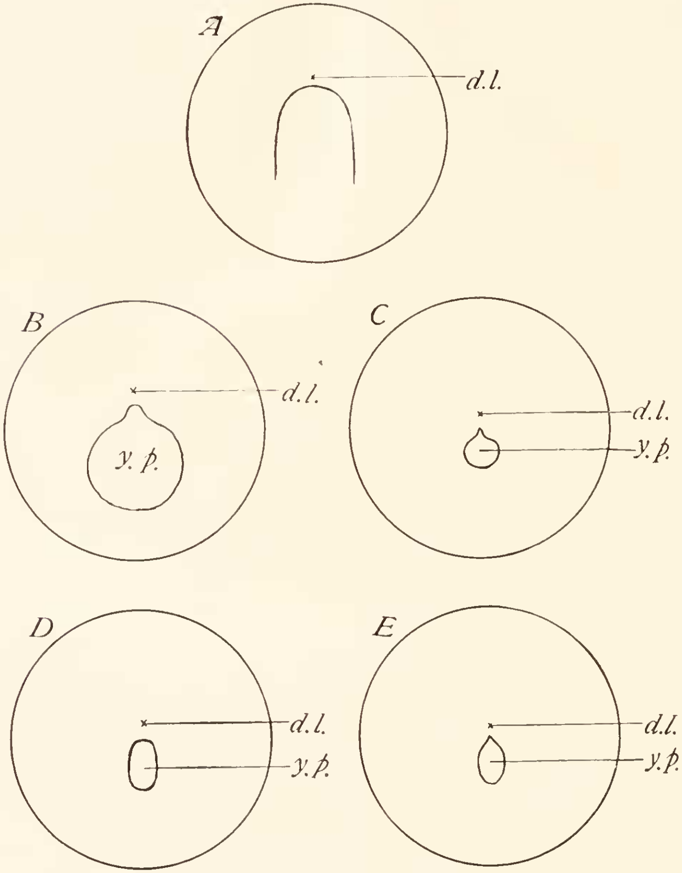

FIG. 16. Inhibition of dorsal lip region. Eggs placed in $m/10$ LiCl in late cleavage stages immediately before gastrulation. *A* and *B* after 18 hrs. in the solution; *C*, *D* and *E* after 54 hrs. in the solution. *d.l.*, dorsal lip region; *y.p.*, yolk plug. (Experiment IV 76.)

varies from nearly normal (in the less inhibited ones) to 10/10 (more inhibited ones). In the more extreme inhibitions some cases of secondary invaginations are seen and a decrease in the gastrular angle is characteristic. Almost any type may be produced at will simply by varying the concentration of the inhibiting agent, or the length of the exposure, or the stage at which the egg is introduced into the experiment, or by varying several of the factors at once. The chief limiting factor in the control of these and other modifications is the variation in susceptibility exhibited by the eggs of different females. Several preliminary attempts may be necessary before the desired result is obtained. Some of the experiments are cited below, in which these modifications were prominent, and which serve to illustrate a few of the many different ways in which these abnormalities may be produced.

Experiment IV 21.[1] Unsegmented eggs, introduced into $m/7$ LiCl 1 1/2 hours after fertilization. After 21 hours in LiCl and 7 hours in water a few of the eggs showed a slight equatorial wrinkling but no distinct blastopore was present. After 24 hours in LiCl, and 24 hours in water most of the eggs were in late blastula stages in which the segmentation cavity was completely lined with one or more layers of small round slightly pigmented cells.

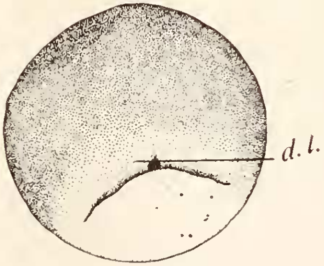

FIG. 17. Inhibition of both dorsal and lateral lip regions of the blastophore, resulting in a "flat-crescent" blastopore. 28 1/2 hours' exposure to $m/10.62$ LiCl from unsegmented stage. (Exp. IV 22.)

Experiment IV 21. After 20 hours in $m/8.5$ LiCl and 8 hours

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[1] Eggs used in experiments IV 21, 22, 26, 27, 29, 31, 33, 34, all came from the same female, and were introduced into the different experiments 1 1/2 hours after deposition.

in water, about 80 per cent. of the eggs were beginning equatorial gastrulation. After 20 hours in this solution and 22 hours in water, about 70 per cent. of the eggs showed various stages of equatorial gastrulation; the rest of the eggs varied from nearly normal yolk plug stages to those showing flat-crescent blastopores (Fig. 17). After 76 hours in water following 20 hours' exposure to the solution about 5 per cent. of the eggs showed a secondary invagination apical to the equatorial blastopore. This secondary invagination appeared more prominently (in about 40 per cent. to 50 per cent.) after 28 hours in *m*/8.5 LiCl and 48 hours in water (Fig. 18).

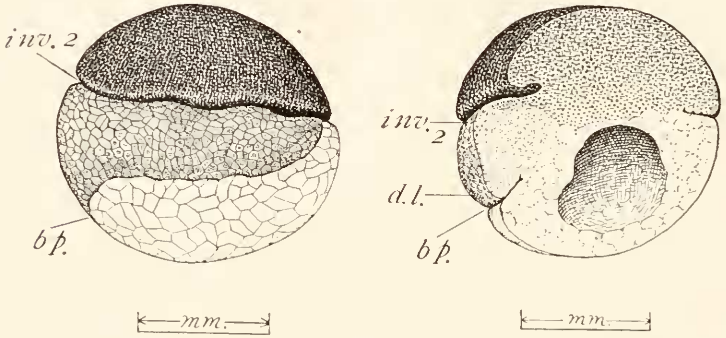

FIG. 18. Secondary invagination following 48 hours' exposure to *m*/10 LiCl, from an unsegmented stage. *A*, surface view; *B*, sagittal section of same egg. ***inv.*** 2, secondary invagination; *d.l.*, dorsal lip; *bp.* blastopore; *s.c.*, segmentation cavity. (Exp. LiCl 1 d.)

Experiment IV 22. 48 hours' exposure to *m*/10.62 LiCl gave 90 per cent. equatorial gastrulæ (Fig. 19), which when returned to water proceeded to develop farther and after 28 hours were in elongated neural fold stages with large protruding yolk plugs. After 72 hours in water following 48 hours' exposure to the solution one finds all stages from a few equatorial gastrulæ to microcephalic and anencephalic forms with partially or completely "fused" suckers, nasal pits, and optic vesicles. After 76 hours' exposure to the solution and 20 hours in water the abnormalities were more extreme. In about 5 per cent. of these the pigmented cells grew out from one to ten mm. to form a cone of cells radially symmetrical about the original polar axis of the egg, there being not the slightest external evidence of bilateriality or dorsoventrality (Fig. 20).

Unsegmented eggs exposed 48 hours to $m/1{,}000{,}000\ HgCl_2$ exhibited all stages between equatorial gastrulation and fairly normal yolk plug stages. Eggs with a broad crescentic blastopore were most prominent. The eggs died without developing farther.

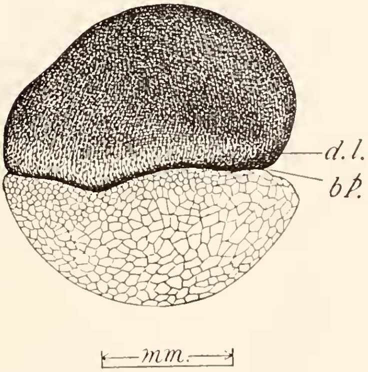

FIG. 19.

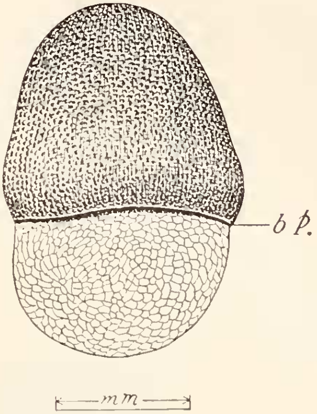

FIG. 20.

FIG. 19. Equatorial gastrulation following 76 hours' exposure to $m/10.62$ LiCl from an unsegmented stage. (Experiment IV 22.3.)

FIG. 20. Embryo radially symmetrical about the polar axis. Produced by exposing unsegmented eggs 76 hrs. to $m/10.62$ LiCl; 20 hrs. in water. (Experiment IV 22 b.)

Experiment IV 34. Eggs in 8-cell stage (10 hours and 20 minutes after deposition) were placed in $m/5{,}000\ KMnO_4$. After 48 hours' exposure—solution not changed—the eggs showed a complete series of stages from perfect equatorial gastrulæ to normal yolk plug stages. At this time all of the permanganate had been reduced to the brown form and the solution had evidently lost much of its toxicity. Sixty hours after being placed in the solution about 50 per cent. of the embryos were elongating but were markedly macrocephalic, showing differential recovery.

(*d*) *Secondary Invaginations.*—The eggs described by this term show a secondary invagination appearing in a meridian bisecting the original blastopore which is usually equatorial. The invagination may extend partly or completely around the egg and appears only under conditions of severe inhibition. In addition to this secondary inturning, some overgrowth may take place; in some cases the over growth is in the direction of the vegetal pole as in normal gastrulation, while in other cases the overgrowth is toward the apical pole. In eggs where this upward overgrowth is taking place over the secondary invagination, the yolk cells below the original blastopore are usually overgrowing the pigmented cells of the animal hemisphere, thus reversing the usual process.

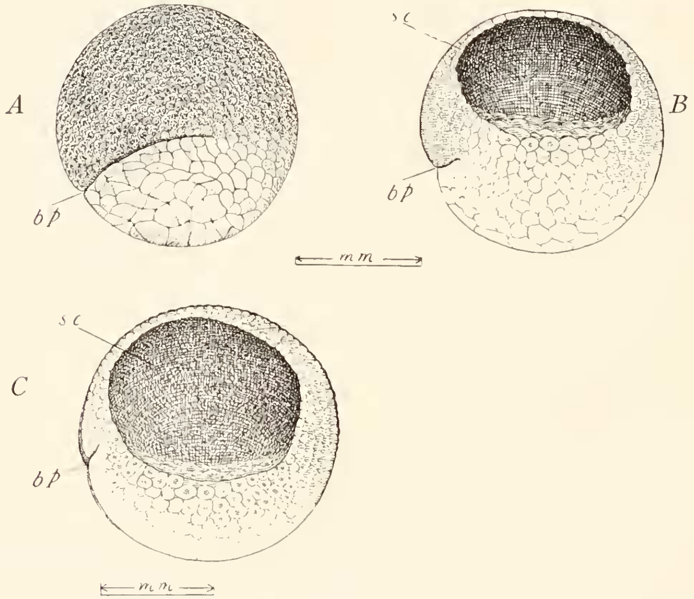

FIG. 21. Some types of modification seen after the following treatment in KNC: *A*, *B* (same egg). Eggs beginning two-cell at start of experiment; 24 hrs. in *m*/1,000, 24 hrs. in *m*/20,000, 12 hrs. in *m*/50,000, 12 hrs. in *m*/100,000. C, 12 hrs. *m*/1,000, 12 hrs. *m*/5,000, 24 hrs. *m*/10,000. (Experiment KNC A 3.) Note the position of the blastopore which is nearer the equator than in control eggs.

This modification is similar to that described by Morgan (1903), which he says is produced by a process in which "the cells of the upper hemisphere . . . turn into the egg." It makes little difference whether one refers to the process thus or whether one calls it simply an invagination. I have used the latter term because the process appears to me to be similar in many respects to that of invagination and overgrowth seen in gastrulation.

Some of the experiments in which this secondary invagination appeared have been cited. They are illustrated in Figs. 14, *C*, and 18. Several other experiments follow.

Experiment LiCl 1. Unsegmented eggs placed in *m*/10. 6 hours: not appreciably different from control. 24 hours: beginning equatorial gastrulation. Pigment has decreased somewhat in the cells of the dorsal lip region. The probable significance of this disappearance of pigment has been mentioned (p. 321). 30 hours: large yolk plug—equatorial gastrulæ. 48 hours: equatorial gastrulæ with secondary invaginations.

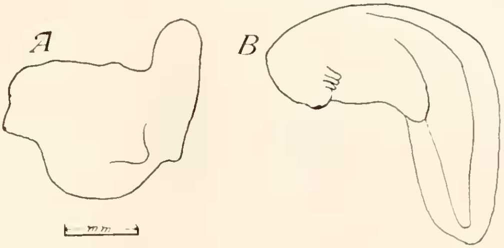

FIG. 22. *A*, differential inhibition—fused suckers, nasal pits, dorsal concavity. Three hours' exposure (from late cleavage) to *m*/5 LiCl; four days in water. (Experiment IV 58 b.) *v.s.*, ventral sucker; *n.p.*, nasal pit. *B*, differetnial recovery. Note the marked dorsal convexity. (Experiment IV 59 e.) Two days' exposure (from late cleavage) to *m*/10 LiCl; five days in water.

In experiment IV 70 (see p. 337) this modification occurred in about 10 per cent. of the eggs following 24 hours' exposure to *m*/500,000 $HgCl_2$ and 24 hours in water. A few cases were noted following 48 hours' exposure, from unsegmented, to 0.0075 per cent. formaldehyde (Experiment IV 83). In experiment R 135, eggs just beginning to gastrulate were placed in 0.001 per cent. formaldehyde and after 24 hours' exposure showed equatorial gastrulæ with secondary invaginations similar to the one shown in Fig. 18. Eggs just beginning to segment when subjected to the following graded treatment showed various degrees of this modification in about 50 per cent. of the eggs: KNC *m*/1,000 24 hours, *m*/20,000 24 hours, *m*/50,000 12 hours, *m*/100,000 12 hours. (Experiment KNC H 5).

(*e*) *The Gastrular Angle.*—In the normal egg the blastopore normally makes its appearance in a meridian that bisects the grey crescent and about 40° below a plane through the center of the egg and parallel to the floor of the segmentation cavity. It is characteristic of strongly inhibited eggs that the gastrular angle approaches and often equals 0. Not infrequently the angle becomes negative, *i.e.*, a circular blastopore is formed *above* the equator. Some of the experiments where this shifting of the point of appearance of the blastopore occurred have been cited (pp. 337, 339, 340). Several variations of this modification are illustrated in Fig. 21.

Under conditions where marked acceleration of development occurs, *e.g.*, in *m*/100,000 KNC; *n*/5,000 to *n*/20,000 HCl[6] (2 c.c. to 5 c.c. of *n*/10 HCl in well water) made up in well water, there is a tendency for the gastrular angle to approximate 45° or even 50°. It is much more difficult to alter the space relation of the blastopore and apical region by acceleration than by inhibition. It may be noted that the effects of acceleration become more apparent about the time of hatching. In these cases the embryos are markedly macrocephalic and dorsally convex, indicating more rapid growth of the anterior and dorsal parts.

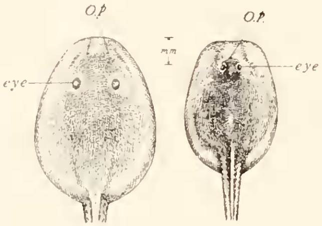

FIG. 23. *A*, normal. *B*, microcephalic, eyes closer together than normal, nasal pits fused, ventral suckers absent. Three hours' exposure (from early gastrula) to *m*/7 LiCl; 13 days in water. (Experiment R 122.1.)

C. *Differential Inhibition in Later Development.*—The whole problem of differential susceptibility involves of course, not only differential inhibition, with which this paper is primarily concerned, but it also includes differential acclimation and recovery,

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[6] I am aware that acid is generally regarded as an inhibitor of protoplasmic activity, but in these experiments the acceleration of development in certain concentrations of HCl is perfectly definite and distinct. Similar results were obtained independently and repeatedly by both Dr. Child and myself. Since the well water in which the solutions were made up contains various salts and other substances in minute amounts it remains to be decided whether the acceleration of development in these cases is a direct or an indirect effect of the acid.

the data on which are fully as striking and significant as those on differential inhibition. But their consideration is reserved for future consideration along with a detailed discussion of differential susceptibility in the later stages of development in the frog's egg. However, for the sake of completeness a few of the more striking abnormalities of later development may be mentioned. These are: microcephalic forms with such bilateral structures as optic vesicles, ventral suckers, and nasal pits in all degrees of approximation from nearly normal to complete "fusion."[1] These forms are seen somewhat more frequently in $m/10$ LiCl than in other agents but they have been obtained in varying percentages in all of the agents used to modify development. Several of the forms are illustrated in Figs. 22–25. The history and treatment of the eggs is given in the legends to the figures.

FIG. 24. Cyclopic frog embryo. (Experiment R 122.1.)

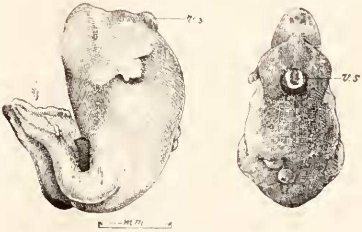

FIG. 25. Spina bifida, microcephalic, ventral suckers nearly fused. Twelve hours' exposure (from early gastrula) to $n/300$ NaOH; four days in water. (Experiment R 125.4.)

In addition to the marked apical inhibitions just mentioned the later stages show also considerable median dorsal inhibition

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[1] The term "fusion" is used in a purely descriptive sense for the eye of cyclopic forms, single median nasal pits, or single median ventral suckers. Whether the primordia of the organs mentioned arise as paired structures and later fuse to form a single median structure, or whether a single primordium arises that later divides into two parts in normal individuals, but which do not separate under strongly inhibiting conditions, is a question for future consideration.

(Fig. 22, *A*) indicated by the marked dorsal convexity with the tail extending upward, in some cases at right angles to the body. The medullary folds may fail to close in the brain region, in the brain region and at the posterior end, or they may not close at all throughout their entire length. In some cases anencephalic forms appear. Spina bifida of all degrees is of course common under conditions that inhibit development, and result primarily from inhibition of the dorsal lip region.

V. DISCUSSION.

It has been shown that gradients in susceptibility to several external agents do exist in the egg and embryo of frog. Concerning the *fact* there can be no question. It was pointed out that the experimentally produced modifications described in this paper are readily and logically accounted for on the basis of differential susceptibility. Now, since no evidence was found of: (*a*) "specificity" in the sense in which the term has been used to explain terata produced by various chemical agents; (*b*) of "blastolysis" or (*c*) of any "disorganization" effect of the chemicals used to modify development as effective factors in the production of terata, the interpretation of the data is now largely a matter of pointing out the basis and significance of differential susceptibility. This is the chief task of the discussion.

1. *The Nature of Physiological Axes.*—These differences in susceptibility to external agents exhibited by different parts of the living egg and embryo (see pp. 322–344) must represent differences of some sort in protoplasmic conditions. Living protoplasm is a complex system of correlated, interdependent reactions and conditions, and it is unquestionably true that different agents affect it differently or attack it at different points. But, whatever the particular factor or factors chiefly concerned in determining these differences in susceptibility to particular agents may be, whether it be rate of fundamental oxidation-reduction processes, state of colloidal aggregation, permeability of limiting membranes, or something else, the general uniformity and lack of specificity in the susceptibility relations to different agents and conditions, not only as regards the egg and embryo of the frog but as regards animals and plants generally, force us to

certain conclusions. These are briefly: (1) that differences in susceptibility must depend primarily upon quantitative differences in protoplasmic condition, rather than upon the specific or qualitative differences, and (2), that these quantitative differences are closely associated with differences in the rate at which fundamental physico-chemical processes are taking place in different regions of an individual or in different indivduals.

These facts, viz., differential susceptibility relations and their marked uniformity and lack of specificity, not only in the frog but also in other organisms; gradients in protoplasm-yolk and pigment distribution both of which parallel the polar axis; and the fact that development begins and proceeds in a definite, orderly, and sequential manner with respect to certain axes (or to the polar axis) in such a way that structural and functional order are at all times referable to one or more axes or planes of symmetry (anterior-posterior, dorso-ventral, medio-lateral), constitute evidence for the existence of a fundamental underlying order of some sort that exists prior to, and which conditions the orderly, sequential, and spatial relations of parts that become apparent later in development. Furthermore it is this order, of which differential susceptibility is one expression, which affords the basis for the great uniformity and lack of specificity in the susceptibility relations, not only in the frog but also in other organisms.

The nature and origin of this underlying order is the problem of physiological axiation—a problem that has been the subject of no little speculation and experimentation. It has been thought by some that polarity and symmetry as manifested in the organism are fundamental properties of protoplasm, and, like the polarity and symmetry of crystalline substances or of the magnet, are intra-molecular in character. It may be said, however, that this conception and others of a similar nature, do not stand the test of experiment even where capable of such an analysis.

On the basis of extensive experimental work Child has arrived at a dynamic conception of physiological axes in the organism with the aid of which he accounts in a satisfactory manner not only for many of the hitherto unrelated facts of normal and abnormal development, but also he has been able experimentally to modify and control development on a predictable basis.[1] Child looks upon a physiological axis in its simplest terms as a quantitative gradation in physiological condition. He has brought forward evidence from a number of sources which point to the conclusion that this gradient is a gradation in rate of metabolism, or at least of certain fundamental reactions such as the oxidation-reduction processes, which is associated with a gradation in the physical condition and constitution of the protoplasm.

If a gradient in physiological condition is a fundamental ordering factor in the process of physiological axiation, it should be possible to obtain as many expressions of this gradient as there are aspects into which fundamental metabolic activity and the physical constitution of the protoplasm may be analyzed. Hence, we might expect to find, *e.g.*, gradients in irritability; in growth and differentiation and development; in the visible morphological condition of the protoplasm; in permeability; in the active mass of enzymes; in electrical potential; in oxidation as measured by oxygen consumption and in carbon dioxide production; and conversely, gradients in susceptibility to lack of oxygen. Well known biological facts afford demonstrative evidence for the existence of some of these gradients. Further evidence for the existence of other of these gradients has been published by Child, Hyman, et al.[1] Other evidence is yet unpublished.

"All the various lines of evidence considered agree in showing that axial gradients in the dynamic processes are characteristic features of organisms and that a definite relation exists in each individual between the direction of the gradient in any axis and the physiological and structural order which arises along that axis. In the major axis the region of highest rate in the metabolic gradient becomes the apical or anterior region of the individual, and in the minor axes also the regions of highest rate in the gradients represent particular features of the order in each case. Along any axis particular parts apparently represent particular levels in the gradient. The variety, extent, and agree-

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[1] See footnote 1, page 315.

ment of the evidence is all the more interesting in view of the fact that such gradients have not heretofore been recognized as characteristic features of organic constitution." (Child, 1915*a*, p. 87.)

2. *Origin of the Dorsal Lip Region.*—The data on differential killing and differential inhibition in the frog show, not only that the apical pole region is more susceptible than other parts of the egg during early development, but also that as development proceeds a secondary region of high susceptibility appears in the sagittal plane about 100° below the apical pole. This is the dorsal lip region. Morgan and Boring (1903) showed that in the eight cell stage, that cell of the upper quartet lying nearest the grey crescent is slightly smaller than the other three cells of the quartet. This inequality persists up to a stage some time after the beginning of gastrulation, cell division being somewhat more rapid here than elsewhere on the egg. It is well known that during early cleavage some of the apical cells migrate or are pushed downward to form a so-called germ ring that lies at first on the equator of the egg and later below it. Correlated with the greater activity of the apical pole cells in the sagittal plane, this migration or pushing is most rapid in this plane, *i.e.*, in that pigmented quadrant the median meridian of which bisects the grey crescent. This displacement of material from the apical pole region is probably not to be conceived as an active migration but as a consequence of the more active cell division and growth in the embryonic meridian. After the materials in this meridian have been displaced a certain distance from the apical pole they seem to get beyond the range of correlation of that region; certainly they become more active and establish the posterior growing region (dorsal lip). At the time of gastrulation some of the material included in the dorsal lip cells is certainly not less than 100° from where it was in the four-cell stage. What this migration of the materials of the apical region toward the equator of the egg amounts to, of course, is a growth in length which is greatest and proceeds most rapidly in the sagittal plane.

This dorsal lip region, which arises secondarily as a rapidly growing posterior region, appears normally at a fixed distance (about 130°) from the apical end of the polar axis, and it is shown that this distance decreases with increasing inhibition (decrease in metabolic rate) of the apical region, and increases slightly with increase in activity (acceleration) of the apical region. The facts suggest at once a definite physiological correlation between the apical region and the region of the dorsal lip which arises secondarily as a posterior region of active growth and differentiation, and which does not appear to differ fundamentally from the posterior growing region which gives rise to the trunk in annelid larvæ (Child, 1917*a*).

From these facts, viz., the secondary origin of the dorsal lip region, its definite spatial relation to the apical region, which may be controlled experimentally, and its appearance at the distal end of a rapidly growing region, the origin of the dorsal lip region offers an interesting parallel to those cases in certain lower animals and plants where new zoöids or new individuals arising by asexual reproduction have been shown to appear as physiological isolations.[1]

In the simple cases of physiological isolation in lower animals

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[1] The rôle of physiological isolation, dominance and subordination in development has been studied by Child and fully stated by him (1915*a*, pp. 88–169) and need not be considered here except to mention some of the pertinent facts. In Child's own words:

"The experimental evidence demonstrate, first the essential independence of the apical region in both plants and animals, and secondly, determination and control by this apical region of the developmental processes at other levels of the major axis of the individual." (1915*a*, p. 125.)

The facts indicate for those forms investigated, that the control of subordinate parts by the dominant region is transmissive rather than transportative in character, and that the range of dominance, *i.e.*, the distance over which the control of the dominant region is effective and beyond which physiological isolation occurs, varies with the relative metabolic rates of the dominant and subordinate parts, and condition of the conducting path; increasing with increase in metabolic rate in the dominant regions and with the specialization of the conducting path, and decreasing with decrease in the metabolic rate of the dominant region, with increase in rate of the subordinate region, and with interference with the conducting path. These four ways in which physiological isolation may occur are summed up by Child, thus:

"It is possible to control and alter experimentally the spatial relations of parts in the individual by altering the length of the metabolic gradient and so the range of dominance. Parts of the individual may come to lie beyond the range of dominance in consequence of increase in size of the whole, of decrease in range and degree of dominance by decrease in the metabolic rate in the dominant region, of decrease in conductivity of the paths of correlation, and of the direct local action of external factors which increase the independence of subordinate parts." (1915*a*, p. 169.)

such, *e.g.*, as the flatworms, it appears that as the body increases in length the posterior region comes to lie beyond the range of dominance (control) of the apical end. As a consequence, the posterior end undergoes much the same changes that it would if physically isolated. It begins to dedifferentiate and its susceptibility increases, *i.e.*, it comes to resemble physiologically younger tissue. If these processes go far enough the posterior region gives rise to a new zoöid which in time may become a new individual.[1]

In the annelids such a region arises early in development but instead of producing zoöids which develop into independent individuals it proceeds to give rise to segments that are reintegrated and brought under control, probably through the specialization of the nervous system. From this point of view the development of the annelid trunk appears to be a succession of incomplete reproduction processes, but which undergo reintegration into a whole consisting of segments.

In the light of all the facts it is difficult to avoid the conclusion that the origin of the dorsal lip region is fundamentally similar to these processes of physiological isolation, since the dorsal lip region arises secondarily at the posterior end of the embryonic area as a region of high susceptibility, increased activity, and rapid growth.

The secondary invaginations afford an interesting bit of evidence in that, with certain degrees of inhibition of the apical region, the range of dominance is decreased and after a first isolation has occurred—a second one may arise nearer the apical end.

3. *The Question of "Specificity" in Teratogeny.*—The word "specificity" as used in the literature on vertebrate teratogeny is difficult of exact definition. In general it has been used to designate some unique reaction of an organism or part, to a particular experimental factor, especially a particular chemical substance, such as to produce an anomaly characteristic of that individual external factor.

Various attempts have been made to identify experimentally produced abnormalities with either a specific chemical effect or with a more general physical effect of some one of numerous

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[1] Child, 1911, *Jour. Exp. Zoöl.*, Vol. 11.

agents that have been used to modify development. The literature of this subject has been reviewed incidentally so many times (Korschelt and Heider, 1902; Hertwig, 1906; Jenkinson, 1909) that only certain essential facts and conclusions need be mentioned here. Gurwitsch (1896) attributed the various abnormalities of the frog produced by him in sodium bromide, lithium chloride, and weak solutions of strychnine, caffein, and nicotine, to an "einigermaassen specifische" action of individual chemicals upon certain regions of the egg. Stockard (1909) at one time believed that cyclopia in *Fundulus* was due to a specific cyclopic producing property of $MgCl_2$ and went so far as to suggest that cyclopia in man may be due to an unusually high amount of Mg in the amniotic fluid or in the blood of the pregnant mother. When, however, it had been shown that a large number of different substances produce the same anomaly, Stockard (1914) abandoned his earlier view that there are "specific responses to the given chemical substances employed" and suggested that since "a certain definite response on the part of the developing organism may be consistently obtained after carefully adjusted treatments with a large number of different substances . . . in certain cases they may serve simply to lower the developmental metabolism and thus prevent or arrest the formation of particular structures." No reason is offered to explain *how* lowering the developmental metabolism may cause the prevention or arrest of the formation of particular structures or *why*, under properly controlled conditions, *particular* regions of the embryo are affected more than other parts and in a definite and sequential relation to one another, though he notes that the nervous system and special sense organs are most affected by the use of chemical agents that inhibit development.

To digress for a moment, in the light of present knowledge we believe it possible to give a rational interpretation of these facts. The reason that a lowering of the developmental metabolism under conditions where acclimation does not occur, can prevent or arrest the formation of such structures as nervous system and special sense organs, is the fact that there is a gradient in the rate at which the processes described by the collective term "metabolism" are taking place along physiological axes. Anterior, medial, dorsal regions, because of their higher metabolic rate are more susceptible to the inhibiting conditions and hence are more affected.

Bataillon (1901) studied the abnormal development of the frog in isotonic solutions of cane sugar, NaCl, and a number of other salts and reached the conclusion that osmotic pressure and the consequent withdrawal of water from the developing embryo, is the effective cause of abnormalities. Jenkinson (1906) showed that solutions of a large number of substances, isotonic with 0.625 per cent. NaCl, do not produce the same, but markedly different effects. It may be pointed out however that these effects differ not in kind, but in degree, and are on the whole readily interpreted in terms of differential susceptibility. And it may be remarked here that the large number of abnormalities in the frog described during the past quarter of a century which have been produced by experimental means may all be rationally interpreted in these terms and readily brought into relation with the axial gradients. Jenkinson's conclusion was that the abnormalities produced—covering most of the modifications known in amphibian teratology—were not to be attributed to the osmotic pressure of the solutions but were due to their chemical or physical properties. Morgan who has done much work on abnormal development in the frog, stated, (1906) in speaking of the osmotic and chemical effect of salt solutions on the frog egg, that: "It is probable that the effect is a double one; in part chemical, in part osmotic."

There can be little doubt that certain substances especially in high concentrations, do have a physical as well as a chemical effect upon protoplasm. Furthermore, in a highly specialized egg like that of the frog where a considerable volume of relatively inert matter (yolk) is localized in one hemisphere, high osmotic pressures such as exist in solutions of certain electrolytes and other substances in concentrations high enough to cause markedly abnormal development, and substances that have a solvent action on the yolk (alcohol, *e.g.*), may in extreme cases set up secondary disturbances of a mechanical sort such as partly or wholly to obscure the general susceptibility relations. But, it is especially to be noted that those agents which, in extreme concentrations or intensities of action, do tend to obscure the differential susceptibility relations, may be used in other concentrations or intensities, to produce differential inhibitions that, in kind, are not different from the differential inhibitions produced in a variety of other agents.

Although it was stated (p. 346) that in the reaction of the frog egg to the different substances used to modify development, no evidence was found of any "specific" action, *in the sense in which the term has been used*, I do not wish to be understood as implying that all chemical substances act upon protoplasm in exactly the same way. It is to be expected, and bio-chemical and zoölogical literature contain abundant evidence to show, that different agents do affect protoplasm in different ways. KNC, *e.g.*, which is a powerful reducing agent is known to interfere with oxidations and enzyme activity in the organism. Hyman (1919) found that in planaria oxygen consumption may be reversibly cut down as much as 80 per cent. to 90 per cent., in the presence of minute amounts of KNC. In this paper she gives a comprehensive review of the literature on the action of cyanides on protoplasm so that further discussion of this matter is unnecessary here. So far as susceptibility relations are concerned formaldehyde appears to affect protoplasm in much the same way as KNC, and $KMnO_4$ which is a vigorous oxidizing agent is equally efficient for demonstrating differences in susceptibility in organisms.[1] It is a significant fact that these oxidizing and reducing agents are among the most effective agents that have been used in the study of physiological axiation.

As regards anesthetic action in the stricter sense, whether these agents disturb oxidations, affect the lipins of the cell, produce changes in the surface membranes, etc., there appears to be no consensus of opinion.

Various salts affect protoplasm, especially the colloid substances, in widely different ways. They may bring about changes in the state of aggregation of colloids, and the loss or addition of water; induce changes in ionization which may result in the precipitation or solution of certain substances in the protoplasm; or upset the electrical equilibrium, and so on.

Acids and alkalies also affect the speed and type of many

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[1] Child, Biol. Bull., 36, 1919.

reactions in protoplasm in a variety of ways depending upon the ionization constant of the acid or alkali, concentration, reactivity of the substances formed by their interaction with protoplasm, etc.

It is obviously impossible here to go into a detailed discussion of the particular types of reaction set up between external agents and living protoplasm. For a resume of this important subject the reader is referred to Mathew's "Physiological Chemistry," especially chapter V., where references to the literature may be found.

In spite of the various factors concerned in the action of external agents upon living protoplasm, the highly significant fact remains that the susceptibility relations to external agents whose action is severe enough to kill within a few hours or to be distinctly toxic show not only a high degree of uniformity for at least a large number of different agents, but also a very definite correlation with metabolic conditions.

As regards permeability of limiting membranes there can be no doubt that it is an important factor in the movement of substances into and out of the cell. But the passage of many substances across the cell membrane in concentrations high enough to kill the protoplasm follows only upon the destruction of, or irreversible changes in the surface membrane and the consequent more or less complete disappearance of its special physiological characteristics. Furthermore, the cell membrane is alive and therefore metabolically active to some extent, and its peculiar properties as regards permeability are dependent upon the fact that it is alive, since they disappear with its death. It is evident then, first, that semi-permeability is more or less closely associated with metabolic conditions and second, that differential susceptibility to external agents cannot be interpreted in terms of permeability as distinct from metabolic conditions.

Experimental analysis has already shown that many of the so-called cases of specific action of external agents are not properly speaking specific effects at all, but actually are differences in degree rather than in kind. And the question may be raised whether at least many other apparently specific effects will not prove to be of the same sort.

Even admitting that different agents do attack the protoplasmic reaction system at different points—as they undoubtedly do—the important point is that in such a complex and intercorrelated physico-chemical reaction system as protoplasm, it is hardly possible to alter any one set of reactions or conditions to any considerable degree, without disturbing the system as a whole. Consequently in subjecting the frog egg or any other developing organism to an agent that in any way inhibits protoplasmic activity to a sufficient degree, it is certainly, in its broader aspects, not a specific, but a *general* or quantitative effect and reaction of the organism to the agent that becomes apparent. It is the striking uniformity of the susceptibility relations of organisms to so many different agents and conditions that forces the conclusion that there must be some fundamental feature of all axiate organisms that is affected in much the same way by different agents. In other words, *it is the existence of a gradient or gradients in metabolic rate, and protoplasmic conditions associated with them along physiological axes in the organism, that determine the uniformity and the susceptibility relations that have been shown to exist, and afford the basis for a rational interpretation of teratological development.*

VI. Summary.

1. In the ovarian egg of the frog there is a definite relation between the polar axis of the egg and its blood supply. In every case the pigmented hemisphere is more richly supplied with arterial blood than the unpigmented hemisphere. It is suggested that polarity arises in response to this external factor, viz., respiratory and nutritive relation of the egg to the parent body. It also seems probable that the localization of the pigment on the egg is in response to the greater oxygen supply over a restricted area. There is no orientation of the ovarian egg to gravity.

It is pointed out that polarity in a number of other organisms arises in response to conditions external to the egg.

2. It is shown that differences in susceptibility exist in different parts of the egg and embryo of the frog. These differences are evidenced in the following ways:

(*a*) By differential death gradients,

(*b*) By differential inhibition, and

(*c*) By differential acclimation and recovery.

3. The two modifications of the susceptibility method described in this paper, (*a*) and (*b*) above, agree in showing that:

(*a*) Those parts of the egg where development first begins and proceeds most rapidly die soonest in lethal concentrations or intensities of external agents and are inhibited most under conditions so severe that acclimation does not occur. Both disintegration processes and inhibition are differential, paralleling the polar axis in early development and other axes that arise later.

(*b*) All of the modifications, produced under different conditions, are essentially similar, differing not in kind but in degree. No evidence was found of "specificity," "blastolysis" or "disorganization" as effective factors in the production of any of the modifications.

(*c*) The experimental data indicate that any type of abnormality may be produced under the influence of any inhibiting agent by controlling the concentration or intensity of action, the length of exposure and the stage in development (physiological condition) of the egg or embryo when exposed.

4. Differential inhibitions, appearing under conditions that prevent acclimation, are evidenced in the following and other ways:

(*a*) By the relatively slower division of the more active animal pole cells with the result that the cell size ratio approaches and may become equal to one.

(*b*) By the retardation or prevention of downward movement of material from the animal hemisphere, which takes place, normally, most rapidly in the sagittal plane, but which under these conditions, is most retarded in this plane.

(*c*) By the retardation of the dorsal lip region with the formation of V-shaped blastopores and oval yolk plugs that may be several times longer in the sagittal plane than broad.

(*d*) By the retardation of both dorsal and lateral lips of the blastopore resulting in the formation of flat-crescent blastopores and a whole series of modifications between this and,

(*e*) Perfect equatorial gastrulæ.

(*f*) By the appearance of a secondary invagination apical to the original blastopore.

(*g*) By the shifting of the blastopore so that it may come to lie at or above the equator of the egg. (Accompanies *d*, *e*, *f*.)

(*h*) By the apparent obliteration of bilaterality, resulting in the formation of embryos radially symmetrical about the original polar axis.

(*i*) In the later embryonic stages a variety of abnormal types appear most of which are later stages of *c*, *d*, *e* and *g*, above. Among them may be mentioned, microcephalic forms with eyes, nasal pits, and ventral suckers in all degrees of approximation to complete "fusion"; anencephalic forms appear, and spina bifida of all degrees are common. Most of the forms show a marked dorsal concavity and the medullary folds may remain open in the brain region or throughout their entire length.

5. Since the dorsal lip region arises at the distal end of a rapidly growing region, and bears a definite spatial relation to the apical region that is experimentally controllable, it is probable that it arises by physiological isolation consequent upon the rapid growth in length, especially in the sagittal plane.

6. The data presented indicate that at the beginning of development, metabolic processes are most rapid at the apical (animal) pole and decrease toward the basal (vegetative) pole.

7. With advance in development a secondary region (dorsal lip) of high susceptibility appears, probably as a physiological isolation and represents a posterior "segmental" region of rapid growth similar in many respects to the posterior growing region of annelid larvæ.

8. It is pointed out that the existence of gradients in metabolic rate along physiological axes in the egg and embryo make possible the great uniformity and lack of specificity shown to exist in the susceptibility relations in the frog and other organisms, and affords the basis for a rational interpretation of teratogeny, not only in the frog but also in vertebrates generally.

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